Application Note
OCT 2024
AN-850-0146

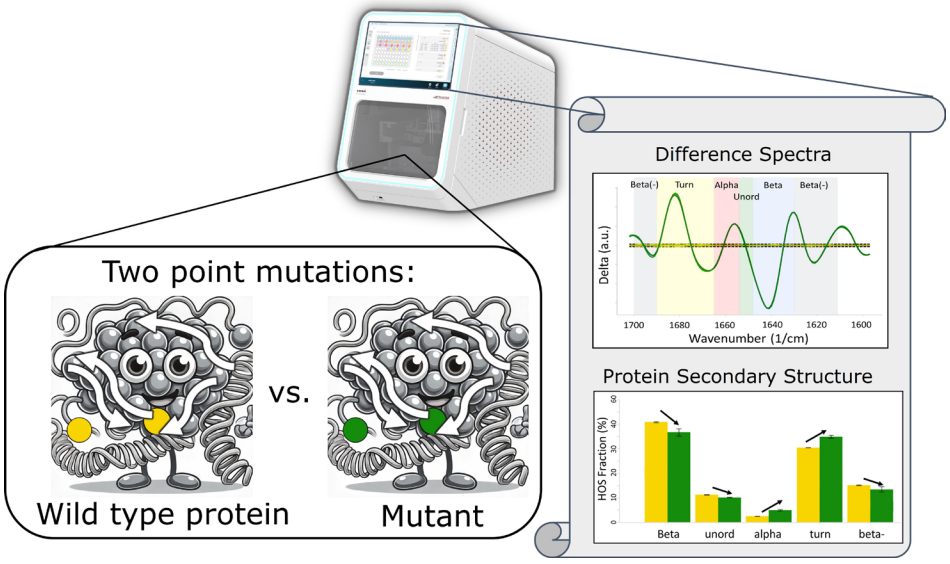
Mutation-induced structural changes of a bacterial protein detected by MMS

- ○ Biosimilars
- ○ mAbs
- ○ ADCs
- ○ AAVs
- ○ Ligand Binding
- ✓ Protein/Peptide Analysis
- ○ VLPs
- ○ Nucleic Acid
- ○ Fusion Proteins
- ○ Enzyme Analysis

- ⬢ Aggregation
- ⬢ Quantitiation
- ✓ Structure
- ✓ Stability
- ✓ Similarity

Abstract

The binding affinity of proteins is directly related to their higher order structure (HOS) since the three-dimensional arrangement of the sequence gives rise to binding pockets and protein-protein/nucleic acid interaction scaffolds. The protein studied in this work is involved in bacterial adhesion and is known to change its binding affinity upon mutation in certain domains. Here, we demonstrate that these mutations are associated with structural changes in the protein which can be successfully identified and characterized using Microfluidic Modulation Spectroscopy (MMS).



Introduction

A key step in urinary tract infection is the adhesion of uropathogenic *Escherichia coli* (*E. coli*) to urothelial cells of the host. This initial step enables the bacteria to invade and colonize host cells but also to withstand clearance by the bulk flow of urine. Our studied protein is a secreted protein involved in bacterial adhesion. Several mutations in one of its domains were linked to a potential decreased pathogenicity as those favored the lower affinity conformational state of the protein promoting bacterial adhesion to host cells. Microfluidic Modulation Spectroscopy (MMS) is known as a very sensitive tool for studying small structural changes in proteins.[1] In this work, we use MMS to study the protein secondary structure of the wild type in comparison to a mutant that only differs by two point-mutations. Previous thermal unfolding experiments have shown that these mutations are associated with a decrease in protein stability. The goal of this study is to demonstrate that the secondary structure of these proteins may serve as a reporter for changes in their affinity and stability.

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Methods

Both the wild type and the mutated proteins were expressed in *E. coli* and purified using affinity and consequent size-based chromatography. The purified proteins at 5 mg/mL were dialyzed in the following buffer recipe: 20mM NaPi pH 7.4, 50mM NaCl. MMS measurements of the dialyzed samples were performed on the Aurora. For background subtraction, the chemically identical buffer taken from the dialysate was loaded pairwise with each sample onto a 96-well plate. The instrument was run at a modulation frequency of 1 Hz and with a microfluidic transmission cell of 23.5 µm optical pathlength. The differential absorbance spectra of the sample against its buffer reference were measured across the amide I band region (1,714 – 1,590 cm^{-1}). For each spectrum, triplicate measurements were collected

Results

The inverted and baseline corrected second derivate spectra of wild type (yellow) and mutant (green) are presented in Figure 1. The triplicate measurements of both proteins showed a high repeatability of 99.8%. An area of overlap analysis[2] of the corresponding spectra reveals a similarity of 87.9%, indicating that the overall spectral difference between wild type and mutant amounts to about 12%. This suggests substantial structural differences between the two proteins, despite the small sequential changes of only two residues.

The bottom part of Figure 1 illustrates the difference spectrum of the mutant compared to the wild type. It indicates that the mutant shows structural differences across all secondary structural components, with the most substantial changes being an increase in turn structure and a decrease in beta sheet. For a quantitative structural analysis, we employed a Gaussian curve fitting model to calculate individual fractions of the corresponding Higher Order Structure (HOS). The results are presented in Figure 2.

As summarized in Figure 2, the 12% overall spectral differences between wild type and mutant reflect structural differences of all secondary structural motifs. We find that the mutant shows decreased fractions of native ("beta", -4.1%) and intermolecular ("beta-", -1.7%) beta sheet, and unordered structure (-1.1%) while the fractions of alpha helical structure (+2.5%) and turn structure (+4.5%) are increased.

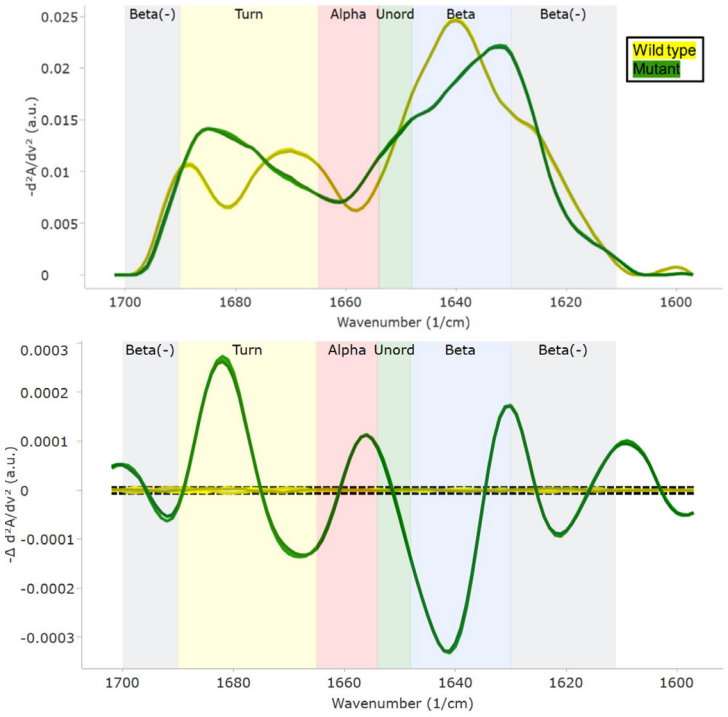

Figure 1: Spectral comparison of the wild type (yellow) against mutant (green) protein within the amide I region, recorded with MMS. Top: inverted and baselined second derivative spectra. Bottom: Difference spectra of the two, using the wild type spectra as the reference. The dashed lines indicate the peak-to-peak noise within the triplicate measurement.

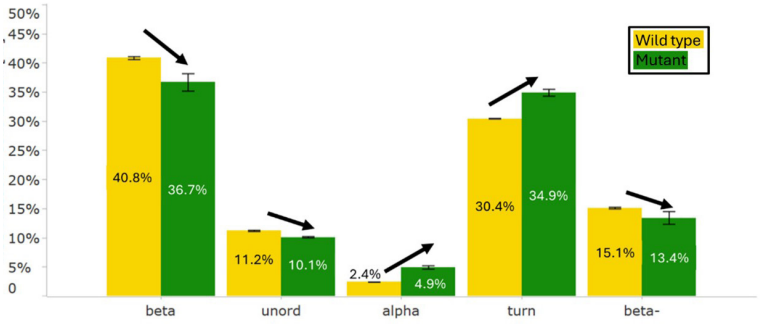

Figure 2: Higher Order structure (HOS) fractional contribution for both proteins, calculated from Gaussian deconvolution of the spectra presented in Figure 1.

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Conclusions

In this work, we have shown that the studied wild type and mutant, differing by only two point-mutations, have substantial differences in secondary structure which gives rise to an overall 12% spectral difference within the amide I band. With a repeatability of >99%, MMS has been demonstrated to serve as the ideal tool for identifying these changes and breaking them down into the individual contributions of various secondary structural motifs. The MMS data reveal that the structural changes induced by the mutation are dominated by a loss in well-folded beta sheet and an increase in turn structure which suggests partial unfolding of the native beta sheet which could lead to a decrease in protein stability. This aligns well with previous thermal unfolding experiments on these proteins which found a lower melting temperature for the mutant. In conclusion, MMS can be used as structural reporter, not only for changes in protein affinity but also stability.

Contributor

Jan Schaefer, PhD

References

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2. Kendrick, B. S., Dong, A., Dean Allison, S., Manning, M. C. & Carpenter, J. F. Quantitation of the Area of Overlap between Second-Derivative Amide I Infrared Spectra To Determine the Structural Similarity of a Protein in Different States. *J Pharm Sci* 85, 155–158 (1996).